SOME ACRIDINE-RESISTANT MUTATIONS OF BACTERIOPHAGE T4D¹

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ABSTRACT

Three new 9-aminoacridine (9AA) resistant mutations of bacteriophage T4D have been isolated and characterized. Two of the mutations, *rs* and *rc*, have identical patterns of acridine resistance, but they map on opposite sides of the *rII* region. In addition, *rs* has an effect on the plaque morphology of *r* mutations, whereas *rc* does not. The third mutation, *ama*, maps very close to *rs* but exhibits a different pattern of resistance to 9AA. None of the three is resistant to acridines by virtue of reduced permeability. Taken together with other mutations that have been previously characterized, these new mutations permit us to set the minimum number of acridine-sensitive processes in T4 development at four.

NINE-AMINOACRIDINE (9AA) has been shown to have several effects on T4 development in *Escherichia coli* (*E. coli*) B/5. Both (1) the time of lysis, as measured by changes in optical density following infection, and (2) the rate of lysis, as defined by the slope of the linear portion of the lysis curve, are affected by the presence of 9AA. The time of lysis is delayed, and the rate is decreased (COUSE 1966 and 1968). (3) Recombination is stimulated in the presence of 9AA, and it has been shown that this is a direct effect of the acridine and not merely a result of the prolongation of the latent period caused by the acridine (MATTSON 1968 and 1970). (4) The overall mutation frequency is increased. In addition certain mutations, such as *rIIUV58* (DRAKE 1963), are especially sensitive to reversion by 9AA (MATTSON 1968). (5) There is also an acridine-sensitive period between 7 and 23 minutes after infection at 30°, known as the "acridine-sensitive clock" (SUSMAN, PIECHOWSKI and RITCHIE 1965). Pulses of 9AA of given duration at any time during the sensitive period cause equivalent delays in the onset of maturation. That is, a pulse from 7–12 min. or 18–23 min. gives the same delay in time of maturation, the actual delay being a function of the concentration of 9AA used for the pulse. (6) 9AA interferes with the gene 17-controlled head finishing step (PIECHOWSKI and SUSMAN 1967). (7) There is also a change in the sedimentation pattern of DNA made in infected cells in the presence of the dye (ALTMAN and LERMAN 1970a,b; RAPPAPORT 1971). Finally, (8) if phages are grown in the presence of a photodynamic acridine such as proflavine, the phages are photoinactivable (HESSLER 1965).

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Physiological studies on the mechanism of T4 production and assembly in the presence of acridines have shown that

- 1) The reduction in the overall amount of DNA synthesized in the presence of acridines is not sufficient to account for the reduction in burst size. The reduction in DNA is not very severe (MANSON 1954; DEMARS 1955). The sedimentation pattern of new DNA as seen on sucrose gradients is altered (ALTMAN and LERMAN 1970b; RAPPAPORT 1971).
- 2) DNA synthesized in the presence of acridine is packaged into mature phage upon removal of the acridine (PIECHOWSKI and SUSMAN 1966).
- 3) Serum-blocking proteins accumulate in acridine-inhibited lysates of infected cells, but maturation is blocked (FOSTER 1948; DEMARS 1955).
- 4) Protein synthesis continues in the presence of acridine (PIECHOWSKI and SUSMAN 1966).

Several acridine-resistant mutants have been isolated. The isolation of two of these, *ac*, an acriflavine-resistant mutant (EDGAR and EPSTEIN 1961) and *q*, a second-step mutation from *ac* (SUSMAN and EDGAR, cited in PRATT, STENT and HARRIMAN 1961) has been previously described. *ama* (McLAREN, unpublished data), *rc* (RUSSEL 1968) and *rs* were selected in this laboratory as second-step mutations from *q*.

We have studied *rs*, *rc* and *ama* in order to elucidate the relationships among these mutations in the hope of defining the number of acridine-sensitive targets in infected cells and their interrelationships.

MATERIALS AND METHODS

Bacteria: Host bacteria were *E. coli* B/5 (EPSTEIN *et al.* 1963). Indicator bacteria were *E. coli* B/5, S/6/5 (*ibid.*), CR63 (APPLEYARD, MCGREGOR and BAIRD 1956), CR63 (λ) and F (λ) (STEINBERG and EDGAR 1962). λ -lysogenic indicators are selective for *rII*⁺ phage. CR63 and CR63 (λ) are *su*⁺_{amber} bacteria. All others used are *su*⁻_{amber}.

Host bacteria were prepared by diluting an overnight culture 1000-fold into 150 ml of fresh medium, incubating with aeration at 30° for 2.5–3 hours, pelleting in the cold and resuspending in 0.5–1 ml of fresh medium. Indicator bacteria were prepared from a 100-fold dilution of an overnight culture into 150 ml of fresh medium incubated and centrifuged as described for host cultures, and resuspended in 15–20 ml of fresh medium. All bacteria were stored on ice or in the refrigerator until use. The normal growth medium was Hershey broth for both host and indicator bacteria.

Phage: The phage used in these experiments were wild-type T4D (DOERMANN and HILL 1953); T4DrI48 (DOERMANN 1952) from EDGAR; T4DrII47; T4DrII73; T4BrIIPB242, transferred into T4D in this lab and T4BrIUV58 (DRAKE 1963), also transferred into T4D in this lab. Also used were the acridine-resistant phages T4DqED41 (PIECHOWSKI and SUSMAN 1967); T4Dac41 (EDGAR and EPSTEIN 1961); T4Dama (McLAREN, unpublished results); T4Drc (RUSSEL 1968) and T4Drs. *ama*, *rc* and *rs* were isolated in this laboratory as second-step mutations from *q*. Many of these phages were used in various combinations of double and triple mutations.

Media: Hershey (H) broth (STEINBERG and EDGAR 1962) was the normal growth medium for bacteria and phage stocks, and was used as the growth medium in most experiments. Tryptone broth was used as dilution medium for titrating phage and bacterial suspension.

M9 or M9CAA was used as growth medium in some experiments. M9 consists of 100 ml 10 × M9 salts, 100 ml 4% glucose, 10 ml 0.1 M MgSO₄, 10 ml 0.01 M CaCl₂, 1.0 ml 1% gelatin and 780 ml sterile distilled H₂O. M9CAA contained 0.25% vitamin-free casamino acids. These

components were autoclaved separately and mixed just before use. $10 \times$ M9 salts are composed of 30 g Na_2HPO_4 , 15 g KH_2PO_4 , 2.5 g NaCl and 5.0 g NH_4Cl dissolved in 500 ml distilled H_2O .

λ buffer was used as dilution and suspension medium in photoinactivation experiments. It consists of 1.36 g KH_2PO_4 and 0.2 g $(\text{NH}_4)_2\text{SO}_4$ dissolved in 1.0 liter H_2O adjusted to pH 7.0 and autoclaved. Just before use it was supplemented to give a final concentration of $10^{-3} M \text{Mg}^{++}$, $10^{-4} M \text{Ca}^{++}$ and $10 \mu\text{g/ml}$ gelatin.

EHA top agar and EHA bottom agar (*ibid.*) were used, respectively, as the top and bottom layers for assays in petri dishes.

Chemicals: 9-aminoacridine (9AA) was purchased from Mann Research Labs, dissolved in sterile distilled H_2O at a concentration of 1.0 mg/ml, and stored in a brown bottle in the refrigerator. Fresh solutions were made monthly. Proflavine and acriflavine, also from Mann Research Labs, were dissolved as above, and stored in foil-wrapped test tubes in the refrigerator for not more than one week. DNase was purchased from Calbiochem, as was 5-bromodeoxyuridine (BrdU).

Preparation of phage stocks: Phage stocks were prepared by adding 0.1 ml of a four-hour plaque resuspended in 1.0 ml of H-broth to 10 ml of H-broth plus 1.0 ml of a B/5 overnight culture and incubating overnight with aeration at room temperature. 1.0 ml of CHCl_3 was added, aeration was continued, and DNase was added to the lysate. The solution was centrifuged for 20 min. at 9000 rpm in a table-top Servall centrifuge to remove bacterial debris.

Standard experimental procedures: Most procedures are specific in RESULTS and DISCUSSION and are standard phage procedures according to ADAMS (1959) and STEINBERG and EDGAR (1962). Experiments involving acriflavine, proflavine, or BrdU were performed either in dim yellow light or with foil-wrapped test tubes. All experiments and incubations were done at 30° unless otherwise specified.

Spot tests: Melted EHA-bottom agar was supplemented with acridine and poured into 100 mm petri dishes. Each dish contained about 30 ml of medium. This layer was allowed to dry at least overnight. Subsequently an overlay of 3 drops of B/5 indicator bacteria in 2.5 ml of EHA-top agar was added and individual plaques were spotted on these plates. The concentrations of dye used to test for various mutants are listed in Table 1.

Photoinactivation experiments: The method is adapted from HESSLER (1965). Bacteria were grown as usual, adjusted to 10^9 cells/ml, and infected with phage at a multiplicity of ~ 5 . The cells were then diluted 100-fold into H-broth containing $0.05 \mu\text{g/ml}$ proflavine. Sixty minutes later the cells were lysed with CHCl_3 and the growth tubes diluted 100-fold into cold λ buffer in glass petri dishes. Under these conditions phage are photosensitized by dye incorporated during growth and not by the small amount of external proflavine in the suspending medium. The lysates were then irradiated with visible light from an unshielded Sylvania Lifeline F40CW

TABLE 1

Acridine concentrations for spot tests

Mutant	Dye	Concentration of dye in bottom layer ($\mu\text{g/ml}$)
<i>q</i>	proflavine	.8
<i>ac</i>	acriflavine	.2
<i>acq</i>	acriflavine	1.5
<i>amaq</i>	9AA	12.0
<i>rcq</i>	9AA	16.0
<i>rsq</i>	9AA	16.0

All of these dye concentrations are restrictive for T4 wild type. Where double mutants are being tested, the dye concentration is restrictive for each of the single mutations. *q* is included in the latter three cases to allow the maturable phage materials to be assembled in the presence of 9AA. See also Table 4.

fluorescent lamp at a distance of 4 cm. Samples were kept on ice during irradiation. Aliquots removed at 0, 10, 20, 40, 60 and 80 minutes and assayed for surviving phage.

RESULTS

We have selected three new 9AA-resistant mutations of T4D. All were isolated by selecting from a stock of *q*ED41 a second-step mutation more 9AA-resistant than the original *q* mutation. These mutants have been named *rs* (*r*-suppressor), *rc* (rapid clock) and *ama* (amino-acridine). These mutations were mapped by standard crosses. The result is shown below.

r-*rs* double mutants exhibit normal *r*-plaque morphology when plated on B/5, but not S/6/5. The plaques on S/6/5 are the same size as *wild-type*, but have sharper edges. The *r*-suppression phenotype of *rs* extends to both *rI* and *rII* point and deletion mutations. The *rs* plaque phenotype was examined by plating on several bacterial hosts. The results are shown in Table 2. It may also be seen from Table 2 that *rs* only affects the plaque morphology of *r* mutations: *rII* mutations, which are normally unable to grow on λ -lysogens, are not enabled to do so by the presence of *rs*. An experiment measuring the O.D. of S/6/5 infected with *r48rs* showed that the altered plaque morphology was not a result of the restoration of lysis inhibition (unpublished results). (Lysis inhibition does not occur in infections with *r* mutations.)

In order to determine if the suppressed-*r* plaque morphology observed on S/6/5 was a result of the T6 resistance of S/6/5, we plated *r73rs* on 13 independently isolated B/5/6 strains. In none of them was the suppressed-*r* plaque morphology observed. Thus it is not merely T6 resistance that determines the plaque morphology difference between B/5 and S/6/5. In crosses done with *rs*, no recombinants separating the plaque phenotype from the acridine-resistance phenotype were observed (unpublished data).

In an additional effort to demonstrate that *rs* consists of a single, pleiotropic

TABLE 2
Plaque morphology of rs

	B/5	S/6/5	Bacterium CR63	CR63(λ)	F(λ)
wild type	+	+	+	+	+
<i>rs</i>	+	+	+	+	+
<i>r48(rI)</i>	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>	<i>r</i>
<i>r48-rs</i>	<i>r</i>	<i>rs</i>	<i>rs</i>	<i>rs</i>	<i>rs</i>
<i>rPB242(rII)</i>	<i>r</i>	<i>r</i>	+	—	—
<i>rPB242-rs</i>	<i>r</i>	<i>rs</i>	+	—	—

The phages indicated were plated on the bacteria shown. Both growth and plaque morphology were scored. *r48* is an *rI* mutation; *rPB242* is an *rII* deletion mutation. *rII* point mutations give results identical to those shown for *rPB242* (unpublished results). + = wild type plaques; *rs* = suppressed-*r* plaques (see text) *r* = *r* plaques; — = no growth.

mutation, rather than two very closely linked mutations, attempts were made to revert either acridine resistance or *r* suppression. *rPB242-rs-q*, a triply-mutant phage carrying an *rII* deletion in addition to the *r* suppressor and *q* was mutagenized with acridines or with BrdU. Ten thousand plaques of this triple mutant on S/6/5 were examined for reversion of *r* suppression and 200 were spot-tested for reversion of *rs*-acridine resistance. No revertants for either phenotype were detected. One *r* plaque arose from BrdU mutagenesis which was still acridine-resistant. However, in crosses to *q* and *rs* both normal *r* and suppressed-*r* acridine-resistant phages segregated. Thus this was not a true revertant, but a new mutation which removed the *r*-suppression characteristic of *rs*. No further studies were made of this mutation except to note that it was linked to neither the *rI* nor *rII* regions. Thus the *r*-suppression phenotype of *rs* is subject to alteration by the genetic background of both the host bacterium and the phage. The basis of this plaque phenotype is still unknown.

Measurements of acridine resistance: As previously mentioned, 9AA has several effects on phage development. We have examined each of these processes in each of the mutants isolated. In most cases the process was examined both for the single mutant and *q* double mutant. This approach should have allowed detection of interactions of *rs*, *rc* or *ama* with *q*. In most cases data are presented only for *rs*. The results for *ama*, *rc*, *rs-q*, *ama-q* and *rc-q* will be described.

Burst size: We have measured burst sizes in the continuous presence of low concentrations of 9AA. This experiment is an overall measurement of both the ability of a phage to make precursor substances such as DNA in 9AA as well as the ability to assemble these precursors into viable phage in the presence of 9AA (PIECHOWSKI and SUSMAN 1966). Thus the ability to overcome 9AA inhibition of early processes and the late gene-17-controlled head finishing step (PIECHOWSKI and SUSMAN 1967) can be examined. Some results are shown in Table 3. *ama* and *rc* give results similar to *rs*; *ama-q* and *rc-q* give results similar to *rs-q* (unpublished results).

Lysis: We have also examined lysis of infected bacteria in the continuous pres-

TABLE 3

Burst size in 9AA

	0	Concentration of 9AA ($\mu\text{g/ml}$)				0 (not normalized)
		.25	.5	1	1.5	
wild type	100	114	49	2.0	.049	205
<i>q</i>	100	83	81	12	1.7	138
<i>rs</i>	100	82	72	8.8	.38	144
<i>rsq</i>	100	120	113	77	82	100

These burst sizes have been normalized to a value of 100 in the absence of 9AA. The actual burst sizes out of 9AA are listed in the last column. B/5 host bacteria at a final concentration of 2×10^8 cells/ml were infected with the indicated phage (m.o.i. 10), adsorption with aeration was continued for 4.5 minutes, antiserum ($k = 2 \text{ min}^{-1}$) was added to remove unadsorbed phage and allowed to act for 3.5 minutes. The cells were then diluted 10^4 -fold into growth tubes containing H- broth plus the desired concentration of 9AA. Incubation with aeration was continued for 120 minutes and CHCl_3 was then added.

ence of 9AA. COUSE (1966, 1968) has shown that 9AA causes a delay in the time of lysis and a reduction in the rate of lysis in T4-infected *E. coli*. The mechanism of 9AA interference with lysis is not understood. Data for *rs* and *wild-type* are shown in Figure 1. One can see that *rs* is resistant to this effect. *ama* is slightly more resistant than *rs*; *rc* is nearly identical to *rs*; *q* has no effect on the results (unpublished results).

Clock: SUSMAN, PIECHOWSKI and RITCHIE (1965) have described an "acridine-sensitive clock" which controls the time of maturation of T4. 9AA delays the time of maturation of T4 if a pulse of 9AA is given at any time from 7 to 23 minutes after infection at 30°. Pulses of equal duration at any time during this period give equal delays in the time of maturation. The actual delay is concentration-dependent. Data for *rs* and wild type are shown in Figure 2. The time of maturation (T_m) is defined by the intercept with the abscissa of the back extrapolate of the linear portion of the premature lysis curve. The T_m 's from Figure 2 are plotted as a function of acridine concentration in Figure 3. *q* is not resistant to this effect of 9AA; *ama* is less resistant than *rs*; *rc* is as resistant as *rs* (unpublished results).

Mutation: Acridines are efficient mutagens. *rUV58* is especially sensitive to

FIGURE 1.—Lysis in 9AA. Host bacteria were B/5. Adsorption was with aeration for 2 min. The infected cells were then diluted 50-fold into growth tubes containing the desired concentration of acridine and T4 antiserum ($K = 12 \text{ min}^{-1}$). The antiserum prevents lysis inhibition by superinfecting phage. O.D.₄₅₀ was read until the cells lysed. A shows the results for *rs* (m.o.i. 9.8, final bacterial concentration 1.3×10^9 cells/ml); B the results for wild type (m.o.i. 10.7, final bacterial concentration 9.7×10^8 cells/ml). (●) = no 9AA; (□) = 1 µg/ml; (▽) = 2 µg/ml; (△) = 3 µg/ml; (○) = 4 µg/ml.

FIGURE 2.—The 9AA-sensitive clock. B/5 host bacteria at the desired concentration were infected with the desired phage. Adsorption was continued for 1 min and antiserum ($K = 3 \text{ min}^{-1}$) was added and allowed to act for 6 min. The infected cells were then diluted 100-fold into H-broth containing the desired concentration of acridine. 16 min later the cells were diluted an additional 100-fold into acridine-free medium. 1.0 ml samples were blown into CHCl_3 at the times indicated on the abscissa. The number of phage/infected cell was calculated and is plotted on the ordinate. A shows the results for *rs* (final bacterial concentration 1.1×10^9 cells/ml, m.o.i. 3.2). (●) = no 9AA; (□) = 1 $\mu\text{g/ml}$; (▽) = 2 $\mu\text{g/ml}$; (△) = 4 $\mu\text{g/ml}$; (○) = 6 $\mu\text{g/ml}$; (■) = 8 $\mu\text{g/ml}$; (▼) = 10 $\mu\text{g/ml}$. B shows the results for wild type (final bacterial concentration 8.8×10^8 cells/ml, m.o.i. 4.2). (●) = no 9AA; (□) = .5 $\mu\text{g/ml}$; (▽) = 1 $\mu\text{g/ml}$; (△) = 1.5 $\mu\text{g/ml}$; (○) = 2 $\mu\text{g/ml}$; (■) = 2.5 $\mu\text{g/ml}$; (▼) = 3.5 $\mu\text{g/ml}$.

reversion by 9AA (DRAKE 1963; MATTSON 1968). By using increasing concentrations of 9AA one can increase the reversion frequency of *rUV58* from a background of $\sim 10^{-7}$ to $\sim 10^{-3}$. We have looked at the reversion of *rUV58* in combination with *rs*, *rc* and *ama* as double mutants; with *q* as a double mutant and with *q* and the other acridine-resistant mutants as triple mutants. Data for *rUV58-rs* are shown in Figure 4. *rUV58-rc*, *rUV58-rs-q* and *rUV58-rc-q* give results identical to those shown for *rUV58-rs*; *rUV58-ama*, *rUV58-ama-q* and *rUV58-q* give results identical to those shown for *rUV58* (unpublished results).

Recombination: In addition to acting as a mutagen, 9AA stimulates recombination in T4 (MATTSON 1970). We have performed crosses of *r47* (*rII* A cistron) by *r73* (*rII* B cistron) and scored r^+ recombinants. We have done this with and without *q* present, as well as with *ama*, *rs* and *rc*. The data shown in Figure 5 demonstrate the resistance of *rs* to this effect of 9AA. Crosses in which parents carry *rc* give results identical to *rs*; *ama* and *q* give results identical to wild type.

DNA synthesis: ALTMAN and LERMAN (1970a,b) have reported an effect of

FIGURE 3.—Time of maturation in 9AA. These graphs are derived from the data shown in Figure 2. The values for T_m were read from the abscissa in Figure 2. They are plotted here against acridine concentration. A shows data for *rs*, B data for wild type. Note that the scale of the abscissa in A is twice that in B.

9AA on DNA synthesis in T4. They have examined on sucrose gradients the DNA produced during infection in the presence and absence of 9AA, and have demonstrated an effect of 9AA on the pattern thus obtained. We have performed similar experiments using *rc*, *rs*, *ama*, *q*, *rc-q*, *ama-q* and wild-type T4 (RAPPAPORT 1971). ALTMAN and LERMAN observed four components which are defined as follows for a 42 fraction sucrose gradient:

- bottom component—first five fractions—a membrane-bound fraction with sedimentation coefficient greater than 950 S.
- phage component—next 12–15 fractions—mature phage particles with sedimentation coefficient of about 950 S.
- fast component—next 12–15 fractions—170–650 S DNA.
- slow component—remaining fractions—60 S (whole phage size) DNA.

Our results on sedimentation of DNA from phage-infected cells treated with 9AA were as follows (RAPPAPORT 1971):

- wild type and *q* show identical profiles in 9AA—both phage and fast component are missing.

FIGURE 4.—Reversion of *rUV58* in 9AA. B/5 host bacteria, final concentration 5.9×10^8 cells/ml, were infected with the desired phage. Adsorption was continued for 1.5 min. Antiserum was added ($K = 3 \text{ min}^{-1}$) and allowed to act for 3.5 minutes. The infected cells were diluted 20-fold into growth tubes containing H-broth plus the desired concentration of 9AA. 25 min later the cells were diluted 100-fold into acridine-free medium. Growth was continued for 60 min when CHCl_3 was added. Total phage were assayed on S/6/5, r^+ revertants on CR63(λ). (●) = values for *rUV58* (m.o.i. 6.6); (○) = values for *rUV58 rs* (m.o.i. 5).

- 2) *ama*, *rc* and *rs* all show much more fast component than wild type or *q*, but show little if any phage component.
- 3) *rc-q* and *ama-q* (and presumably also *rs-q*) show fast component profiles similar to those they show without *q*, but also show some phage component.

Permeability: All of the effects so far discussed have been tested with 9AA, rather than acriflavine or proflavine (two other acridines). An acriflavine-resistant mutant (*ac*; EDGAR and EPSTEIN 1961) has been previously described which seems to be resistant by virtue of decreased permeability to acridines. This has been demonstrated with both fluorescence measurements (SILVER 1965) of acriflavine uptake and photoinactivation measurements (KAUFMANN and HIATT 1959; HESSLER 1965) with proflavine. (Some of these measurements were actually done with T2 *pr. pr* and *ac* are homologous genes (SUSMAN, unpublished results).) Since 9AA uptake is not amenable to fluorescence measurements (un-

FIGURE 5.—Recombination in 9AA. B/5 host bacteria, final concentration 5.3×10^8 cells/ml, were infected with a mixture of the desired phages. Adsorption was continued for 1.5 min and antiserum ($K = 3 \text{ min}^{-1}$) was added and allowed to act for 4.5 min. The infected cells were diluted 100-fold into growth tubes containing H-broth plus the desired concentration of 9AA. 24 min later the cells were diluted an additional 100-fold into acridine-free media. Growth was continued for 60 min, when CHCl_3 was added. Total phage were assayed on S/6/5, r^+ recombinants on CR63 (λ). (●) = values for crosses of $r47 \times r73$ (total m.o.i. 10, ratio of $r47:r73$ in phage mix 1:2). (○) = values for crosses of $r47 \text{ rs} \times r73 \text{ rs}$ (total m.o.i. 11.2, ratio of $r47 \text{ rs}:r73 \text{ rs}$ in phage mix 1:1).

published results) and 9AA is not a photoinactivating acridine, the resistance of these phages to acriflavine and proflavine was determined. The results are shown in Table 4. Proflavine is the only dye to which all of the mutants were resistant. Therefore it was used in photoinactivation to determine if any of the mutants seemed to be resistant by virtue of decreased acridine permeability. The results are shown in Figure 6. Only *ac*-infected bacteria show decreased permeability to proflavine when compared to wild-type infected bacteria. We can therefore conclude that *rc*, *rs*, *ama* and *q* affect intracellular processes in phage development.

Dominance of rs: We have examined dominance of *rs* in mixed infections of

TABLE 4
Resistance of mutants to different acridines

Mutant	9AA	Acriflavine	Proflavine
<i>ac</i>	0	+	+
<i>q</i>	+	+	+
<i>ama</i>	+	0	+
<i>rc</i>	+	0	+
<i>rs</i>	+	0	+

+ indicates resistance, as compared to wild type, 0 indicates sensitivity equal to wild type. Spot tests were done as described in MATERIALS AND METHODS.

FIGURE 6.—Photoinactivation with proflavine. The experimental procedure is described in MATERIALS AND METHODS. The data are from several experiments, all of which include *rUV58* as the wild type control. Slopes of curves in different experiments were adjusted so that the slope of *UV58* in the presence of acridine was constant. B/5 host bacteria at an average final concentration of 4.8×10^8 cells/ml were used. Proflavine was used at a concentration $0.05 \mu\text{g/ml}$. ($\bullet$) = values for *rUV58*, no proflavine. All other phages used give identical results in the absence of dye (unpublished results). ($\square$) = *rUV58* in dye (m.o.i. 3.3); (∇) = *rUV58 rs* in dye (m.o.i. 2.5); ($\triangle$) = *rUV58 ac* in dye (m.o.i. 5.3); ($\circ$) = *rUV58 rc* in dye (m.o.i. 6.9); ($\blacksquare$) = *rUV58 ama* in dye (m.o.i. 11.2). *rUV58* is present for reasons not relevant to this experiment; the response of *UV58* cannot be distinguished from that of wild type (unpublished results).

mutant and wild-type for sensitivity to 9AA-induced reversion (Figure 7), recombination, or reduction in burst size. In all cases we found intermediate acridine sensitivity (Figure 7 and unpublished results). Thus *rs* and wild type are codominant.

DISCUSSION

A general summary of the data is presented in Table 5. One can see that, as previously reported (SILVER 1965; HESSLER 1965), *ac* (or *pr* in T2) reduces cell permeability to proflavine. *ac* is not 9AA-resistant; so it presumably does not reduce permeability to 9AA. *q* also has been previously described (PIECHOWSKI and SUSMAN 1967). *q* maps in gene 17 and seems to render only the late head-finishing process controlled by gene 17 resistant to 9AA. We see no effect of *q* on any facet of 9AA resistance except the ability to produce a larger burst of phage in the presence of acridine. *q* and wild type show equal acridine sensitivity with respect to all other acridine-sensitive processes examined. This is compatible with the fact that the other acridine effects are produced by early exposure to acridines—from about 6–20 minutes after infection at 30° (COUSE 1966; MATTSON 1968; PIECHOWSKI and SUSMAN 1966, 1967; SUSMAN, PIECHOWSKI and

FIGURE 7.—Dominance: reversion in a mixed infection. The experimental procedure was that described for Figure 4. B/5 host bacteria were used at a final concentration of 6.3×10^8 cells/ml. Indicator bacteria were as for Figure 4. (●) = values for rUV58 (m.o.i. 8.2); (○) = rUV58 *rs* (m.o.i. 5.5); (half-closed circles) = mixed infection with both of these phages (total m.o.i. 6.9, ratio of rUV58:rUV58 *rs* in the input phage 1.5:1).

TABLE 5

Patterns of resistance of five acridine-resistant mutants

	Phage assembly	Clock	Lysis	Stimulation of Mutation	Recombination	Photoinactivation	DNA
wild type	—	—	—	—	—	—	—
<i>q</i>	+++	—	—	—	—	—	—
<i>rs</i>	+	++	+	+	+	—	+
<i>rc</i>	+	++	+	+	+	—	+
<i>ama</i>	+	+	++	—	—	—	+
<i>ac</i>	nt	nt	nt	nt	nt	+	nt

+ = resistant — = sensitive nt = not testable

All processes except photoinactivation are tested with 9AA, to which *ac* is not resistant. Photoinactivation is tested with proflavine, to which all of the mutants are resistant.

RITCHIE 1965)—while gene 17 controls a later step in phage development (PITCHOWSKI and SUSMAN 1967). Presumably the other mutants affect burst size by increasing the amount of precursors produced in the presence of acridine. These precursors are then assembled into phage with low, wild-type efficiency rather than *q* efficiency.

ama, *rc* and *rs* divide the early acridine-sensitive processes into at least two groups. Since *ama* is not resistant to the effect of 9AA on mutation and recombination, these effects are in some way distinct from the effects on lysis, time of maturation, and DNA synthesis to which *ama* is resistant. A distinction between these processes is indicated by other sorts of evidence as well. One can use the data presented in RESULTS to calculate the factor by which *rs* increases acridine resistance. This has been done for lysis, time of maturation, reversion and recombination. Acridine dosage for *rs* must be multiplied by a factor of about 4 or 5 to produce delays in maturation or lysis equal to wild type. For recombination and reversion, however, this factor is about 8–10. *rc* gives the same results (RUSSEL 1968).

Using the data presented one can estimate the minimum number of acridine-sensitive “targets” in T4 development. (1) There is one group of 9AA-sensitive processes (clock, lysis, fast component DNA) protected by *rc*, *rs* and *ama*. (2) There is a second group (mutation and recombination) protected by *rc* and *rs*. (3) There is a late assembly step protected by *q*. (4) There is a permeability barrier to some acridines that is influenced by *ac*. There are also indications that further distinctions can be made between these processes. *rc*, *rs* and *ama* are all resistant to inhibition of the clock and of lysis, but *rs* and *rc* are more resistant than *ama* to the “clock” effect while *ama* is more resistant to the lysis effects (unpublished results). Furthermore, MATTSON (1968) has shown that concurrent protein synthesis is necessary for 9AA stimulation of recombination, but is not necessary for 9AA stimulation of mutation. Thus it seems likely that the actual number of 9AA targets is greater than four.

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